THE STRUCTURE OF THE DIGESTIVE SYSTEM IN BOLITOTHERUS CORNUTUS.

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Several specimens of the forked fungus beetle (*Bolitotherus cornutus*) were obtained in April from woods in Hancock County, Ohio. At the suggestion of Dr. C. H. Kennedy a study of the internal morphology of this species was undertaken. All of the individuals collected in the spring were scattered underneath the bark of fallen logs on which was growing the large wood fungus (Polyporus). The beetles were in a state of hibernation. When the woods were again visited on July 30, specimens were obtained at the base of the fungus on stumps rather than on logs. The beetles brought into the laboratory in July were much more active than those taken in April. Larvae were present in the fungi both in April and July and although none were reared they were probably the larvae of this fungus beetle. A few eggs were laid in the laboratory by the beetles collected in July.

Bolitotherus cornutus, of the family Tenebrionidae, is common in Northeastern United States and Canada. The whole surface of the body and wing covers are very rough and there are two prominent horns on the prothorax, those on the male being much longer than those on the female.

These individuals are dull black in color and resemble a small piece of bark. They are approximately 9 mm. long and 5 mm. broad.

Specimens were kept alive in the laboratory by feeding them moist pieces of fungus. They were studied immediately after killing and the material which was to be used for sections was fixed with Kahle's fixative fluid as soon as the parts could be dissected in normal salt solution. Sections were cut from 6–10 microns in thickness. Some were stained with haemalum and fast green, others with Ehrlich's hematoxylin and Biebrich's Scarlet. The chitinous linings, cell walls, and cytoplasm were clearly stained by the fast green. The haemalum proved to be an excellent nuclear stain for this beetle.

The author wishes to express her thanks for the helpful suggestions and criticisms of Dr. C. H. Kennedy, under whose direction this study was made, also to fellow students for assistance in collecting and for suggestions in laboratory methods.

GROSS ANATOMY OF THE DIGESTIVE TRACT.

General Discussion.—The alimentary canal of *Bolitotherus cornutus* is a somewhat convoluted tube of approximately 25 mm. in length, that is almost three times the length of the beetle itself. It is divided into three chief regions, the fore-intestine (Stomodaeum), mid-intestine (Mesenteron or Ventriculus), and the hind-intestine (Proctodaeum).

The Gross Structure of the Fore-Intestine.—The fore-intestine is very short, being 3 mm. in length. It is composed of the pharynx, oesophagus, crop and oesophageal valve. (Plate I, Fig. 1.)

The *pharynx* extends from the mouth as a straight tube, having muscle attachments radiating out from its surface and connecting it with the head capsule.

The oesophagus continues as a narrow tube from the pharynx and terminates by a slight enlargement, the crop, in the center of the prothorax.

The oesophageal valve, a slight constriction marking the end of the fore-intestine and the beginning of the mid-intestine, is for the most part hidden by a wide circle of pipilliform crypts at the front end of the stomach. These crypts are longer than those found further back on the mid-intestine. (Plate I, Fig. 1.)

No salivary glands were found.

The Gross Structure of the Mid-Intestine.—The mid-intestine, or stomach, comprises the largest part of the alimentary tract, being 12 mm. in length. (Plate I, Fig. 1.) It extends from the oesophageal valve posteriorly as a straight tube for a distance of about 7 mm. Here it is 2 mm. in diameter and tapers for the rest of its length, being about ½ mm. in diameter for its posterior 5 mm. It is somewhat convoluted, bending upon itself and then extending cephalad. It is terminated by the pyloric valve and the malpighian tubules. There is a very interesting arrangement of the pipilliform crypts on the surface of the mid-intestine. Following the wide circle of crypts at the extreme front end of the stomach, there are six longitudinal rows for the anterior 7 mm. and four longitudinal rows for the remaining posterior 5 mm. In some other species of beetles it is noted that the crypts are scattered irregularly over the surface of the stomach and even in some they do not extend through the muscle layers to the surface.

In one of the specimens which was obtained in April and kept in the laboratory until June, the anterior end of the stomach was infested with fifteen Gregarines. All but one of the individuals examined in August from the July 30th collection were also infested. They were visible as white blotches through the wall of the stomach and quite well outlined against the dark fungal material which had been injested. Is it possible that they aid in the digestion of the woody fungus?

The Gross Structure of the Hind-Intestine.—The hind-intestine is composed of the pyloric valve, malpighian tubules, ileum, colon, and rectum (Plate I, Fig. 1). This portion of the alimentary canal is 10 mm. in length.

The pyloric valve shows as a constriction in the region of the tract where the rows of papillae abruptly stop. It is just posterior to the attachment of the malpighian tubules.

There are six malpighian tubules, arising from the cephalic end of the ileum and extending forward, dorsally, ventrally, and laterally into the thoracic region; here they bend backward and extend posteriorly to the colon, entering this just underneath the "peritoneal" covering and passing to the end of the colon, where they suddenly disappear. These tubules are visible through the "peritoneal" membrane and are very much convoluted, so that the posterior portion of the colon is almost covered by them. (Plate I, Fig. 1.)

The ileum is a narrow thin-walled tube, located between the pyloric valve and the colon. The anterior end is convoluted and approximately the same diameter as the posterior end of the stomach. In some specimens there was a constriction between the ileum and colon due to the position of the food material in the tract, but usually the ileum is found to continue gradually into the colon without any line marking its posterior limit. The colon is a short straight tube leading to the rectum.

The alimentary tract narrows near the anus to form the heavily chitinized rectum. It is 2 mm. in length.

HISTOLOGICAL STRUCTURE OF THE ALIMENTARY CANAL.

Fore-Intestine.—The layers of the oesophagus, as shown by Plate I, Fig. 2, from the lumen outward are as follows: (1) Intima of cuticula of chitin. (2) Epithelium of hypodermal cells. (3) Basement membrane. (4) Longitudinal muscle. (5) Circular muscle, and (6) "peritoneal" membrane.

The intima is secreted by the hypodermal cells and is a non-cellular, thin layer of chitinous material, homologous with the cuticula of the body wall. In this region it has numerous minute, very short, chitinous spines extending into the lumen.

A single layer of epithelial cells which is in wave-like folds throughout the fore-intestine lies next to the intima. The cells have very large nuclei and the basement membrane is evident, though not conspicuous.

The longitudinal muscles are in scattered groups outside the epithelial layer.

A continuous layer of circular muscle fibers surrounds the longitudinal muscles, and numerous nuclei are scattered throughout the fibers.

There are traces of connective tissue ("peritoneal" membrane) on the outermost part of the oesophagus.

The oesophageal valve (Plate I, Figs. 3 and 4) lies at the junction of the fore- and mid-intestine. In this region numerous long crypts extend out from the wall of the digestive tract making a complete circle around it. This valve consists of a rather large fold of the fore-gut

extending into the lumen of the mid-gut. The intima disappears at the region where the folds of the fore-gut proceed anteriorly. A single crypt opening is also noted at this region, reaching outward at the place where the reflected surface of the mid-intestine joins the fore-intestine. (The dense ring of crypts is slightly farther back on the front end of the stomach.) The epithelial cells of the valve folds are quite large and have distinct, large nuclei. It is here that the reversal in position of the longitudinal and circular muscles occurs. The longitudinal muscles lie internal to the circular muscles in the fore-intestine and external to this layer in the mid-intestine.

Mid-Intestine.—The oesophageal valve lies at the anterior end of the mid-intestine, while the pyloric valve is its posterior limit. This is commonly called the stomach and anteriorly it is about twice the diameter of the posterior portion. It is in this region of the alimentary tract that the papillae are arranged in rows, six anterior and four posterior. See Plate I, Figs. 5 and 6, and Plate II, Fig. 7. Large globules of liquid are present in the lumen of the stomach, indicating merocrine secretion (Plate II, Fig. 8).

Histologically the layers of the anterior and posterior stomach are similar. From the lumen outward are noted the epithelial cells. The layer is one cell in thickness. The striated border resembling cilia is visible on some of these epithelial cells, although those cells which have globules of secreted material at their ends, show no such border. The basement membrane, at the base of the epithelial cells, is not very distinct. A rather thick circular muscle layer is next, and to the outside of this are scattered bundles of longitudinal muscle fibers. A thin "peritoneal" membrane is seen at places covering the longitudinal muscle cells. The nuclei in both the longitudinal and circular muscle layers are quite distinct. There is no intima in this region covering the epithelial cells. Two types of secretion are indicated here, merocrine and holocrine. Merocrine secretion is when the product is secreted by the cells, the latter not being destroyed, while holocrine secretion is when the entire substance of the cells is given up as a secretory product. It appears that holocrine secretion occurs in the long crypts which extend from the surface of the mid-intestine. (Plate I, Fig. 6, Plate II, Fig. 7.) Merocrine secretion takes place at first in the flat inner wall of the stomach, as the globules of secreted material in the lumen show. Later holocrine secretion probably occurs in the epithelial cells of the stomach wall for we find that the nuclei of the cells are very irregular in arrangement, the younger merocrine secreting cells having their nuclei at the base of the cells and the older (holocrine?) secreting cells have the nuclei almost at the end of the cell toward the lumen. (Plate II, Fig. 8.)

The crypts are rounded on the free ends or tips where they contain many nuclei. (Plate I, Fig. 6.) The tip is a nidus.

Hind-Intestine.—The junction of the mid- and hind-intestine is indicated externally by six malpighian tubules which are almost at the level of the last crypts. The tubules come from the hind-intestine and the walls are composed of irregularly shaped cells with large ovate nuclei. There is a lining of intima next to the lumen of the tubules. A delicate "peritoneal" membrane covers the tubules.

The pyloric valve lies just posterior to the attachment of the malpighian tubules. The epithelial cells here are thrown into folds and covered on the lumen side by a lining of intima. There is a thick layer of circular muscle fibers extending the length of the valve. On each side of the circular muscles are scattered groups of longitudinal muscle cells. The exterior layer of longitudinal muscles is almost absent in the region of the pyloric valve. The valvular condition is due to the very thick circular muscle layer at the region where the valve makes a bend. (Plate II, Figs. 9 and 10.)

The hind-intestine begins at the end of the pyloric valve and continues to the anus. The general arrangement of the cell layers is practically the same in the three regions of the hind-intestine, there being an inner lining of intima of cuticula, an epithelial layer, a circular muscle layer, and a longitudinal muscle layer. The intima and epithelial cells are thrown into many folds. (Plate II, Fig. 11.) The cells of the epithelial layer are large, rather cuboidal in shape, and possess very large nuclei located usually at the base of the cells. The circular muscle layer forms a continuous layer, while the longitudinal layer is composed of scattered groups of cells. These muscles are all striated.

A section of the colon (Plate II, Fig. 12) shows the continuous circular muscle layer dipping to touch the intima between the epithelial cells which are arranged in six groups. The longitudinal muscles are also in groups lying between the malpighian tubules. (Plate II, Fig. 13.)

The muscle wall of the rectum is very heavy and is made up largely of circular muscle tissue with a few groups of longitudinal muscle cells scattered near the surface. (Plate II, Fig. 14.) The epithelial cells are smaller than those in the more anterior part of the hind-intestine and they, with the intima, are folded into six distinct groups, known as the rectal pads.

The intima is much thicker here than in the colon. There is a "peritoneal" membrane in this region similar to that in other parts of the tract.

A pair of enlarged anal glands lie in the posterior abdominal region in front of and above the rectum. They are somewhat oval in shape, having a mushroom-shaped cap of secreting cells. Posterior to this cap is the thin-walled reservoir. The reservoirs of the right and left glands unite posteriorly and widen to form the excretory canal. The glands and reservoirs are yellowish in color, due to secreted material in them. According to Bordas (1899) the anal glands function as organs of defense; the yellow fluid secreted by them becomes volatile in contact with air and is of a pungent and acid nature. No histological study was made of these structures, but the manner of attachment of the cap to the reservoir and the connection of the gland to the outside are points quite worthy of further investigation.

BIBLIOGRAPHY.

Berlese, A. 1909. Gli Insetti, 1: 721–757.

Becton, Edward M., Jr. 1930. The Alimentary Canal of *Phanaeus vindex* Macl. (Scarabeidae). Ohio Jour. Sci., 30: 315–323, 2 Pls.

Bigham, John T. 1930. The Alimentary Canal of *Asaphes memnonius* Hbst. Thesis. Ohio State Univ., pp. 1–21, 2 Pls.

Bordas, L. 1899. Anatomie de glandes anales des Coleopteres appartenant a la tribu des Brachininae. Zool. Anz., 22: 73–76.
Bordas, L. 1899. Les glandes defensives ou glandes anales der Coleopteres. Ann. Fac. Sci. Marseille, 9: 205–249, 2 Pls.
Bordas, L. 1901. Morphologie de l'appareil digestif des Dystiscides. C. R. Acad. Sci. Paris, 132: 1580–1582.
Bordas, L. 1902. Variations morphologiques et anatomiques presentees par le gesier chez quelques Coleopteres. C. R. Acad. Sci. Paris, 135: 982–984.
Bordas, L. 1903. L'appareil digestif des Silphidae. C. R. Acad. Sci. Paris, 137: 344–346.
Bordas, L. 1904. Anatomie et structure histologique du tube digestif de l'*Hydrophilus piceus* L. et de l'*Hydrous caraboides* L. C. R. Soc. Biol. Paris, 56: 1100–1102.
Bordas, L. 1906. L'Ampoula rectale des Dystiscides. C. R. Soc. Biol. Paris, 61: 503–505.
Bordas, L. 1906. Morphologie de l'Appariel digestif de l'Anthonome, du Pommier (larve et adult). Bull. Soc. Zool. France, 31: 121–123.
Burgess, Emory D. 1932. A comparison of the alimentary canals of the active and hibernating adults of the Mexican bean beetle *Epilachna corrupta* Muls. Ohio Jour. Sci., 32: 249–261, 2 Pls., 2 Figs.
Davidson, Ralph Howard. 1931. The Alimentary canal of *Criocerus asparagi* Linn. Ohio Jour. Sci., 31: 396–405, 2 Pls.
Deegener, P. 1902. Anmerkung zum Bau der Regenerationscrypten des Mitteldarmes von Hydrophilus. Zool. Anz., 25: 273–275.
Faussek, V. 1887. Beitrage zur Histologie des Darmkanals der Insecten. Zeit. Wiss. Zool., 45: 694–712, 1 Pl.
Fernald, H. T. 1890. Rectal glands in Coleoptera. Amer. Nat., 24: 100–101.
Fletcher, Fred Walker. 1929. The Alimentary canal of *Phyllophaga gracilis* Burm. Ohio Jour. Sci., 30: 109–119, 2 Pls.
Frenzel, I. 1882. Der Verdauungstractus der Larve des *Tenebrio molitor*. Zool. Anz., 5: 215–217.
Korotneff, A. 1894. Zur Entwicklung des Mitteldarmes bei den Arthropoden. Biol. Centralb., 14: 433–434.
Potts, Samuel F. 1927. The Alimentary canal of the Mexican Bean Beetle. Ohio Jour. Sci., 27: 127–137, 2 Pls.
Rengel, C. 1898. Uber die periodische Abstossung und Neubildung des gesammten Mitteldarmepithels bei Hydrophilus, Hydrous und Hydrobius. Zeit. Wiss. Zool., 63: 440–455, 1 Pl.
Schaffer, Paul Everett. 1931. The Alimentary Canal of *Sphaeroderus nitidicollis* Chev. Var. Schaumi Chd. (Coleoptera). Ohio Jour. Sci., 31: 406–415, 3 Pls.
Swingle, Millard C. 1930. Anatomy and Physiology of the Digestive Tract of the Japanese Beetle. Jour. Agr. Res. 41: 181–196. 4 Pls.
Talbot, Mary. 1928. The structure of the digestive system in *Creophilus villosis*. Ohio Jour. Sci., 28: 261–268, 2 Pls.
Wigglesworth, V. B. 1932. On the function of the so-called "Rectal Glands" of Insects. Quart. Jour. Micro. Sci. 75: 131–150, 2 Figs.

ABBREVIATIONS FOR PLATES.

AN. GL...Anal gland.
B. M.....Basement membrane.
C. M.....Circular muscle.
CR.....Crypt.
EPI.....Epithelium.
IL.....Ileum.
IN.....Intima.
L. M.....Longitudinal muscle.
LU.....Lumen.
MT.....Malpighian tubules.
N.....Nucleus.
NI.....Nidus.
OES.....Oesophagus.
OES. V...Oesophageal valve.
PTL. M...Peritoneal membrane.
P. V.....Pyloric valve.
RE.....Rectum.
R. P.....Rectal pad.
ST.....Stomach.
VAL.....Valve.

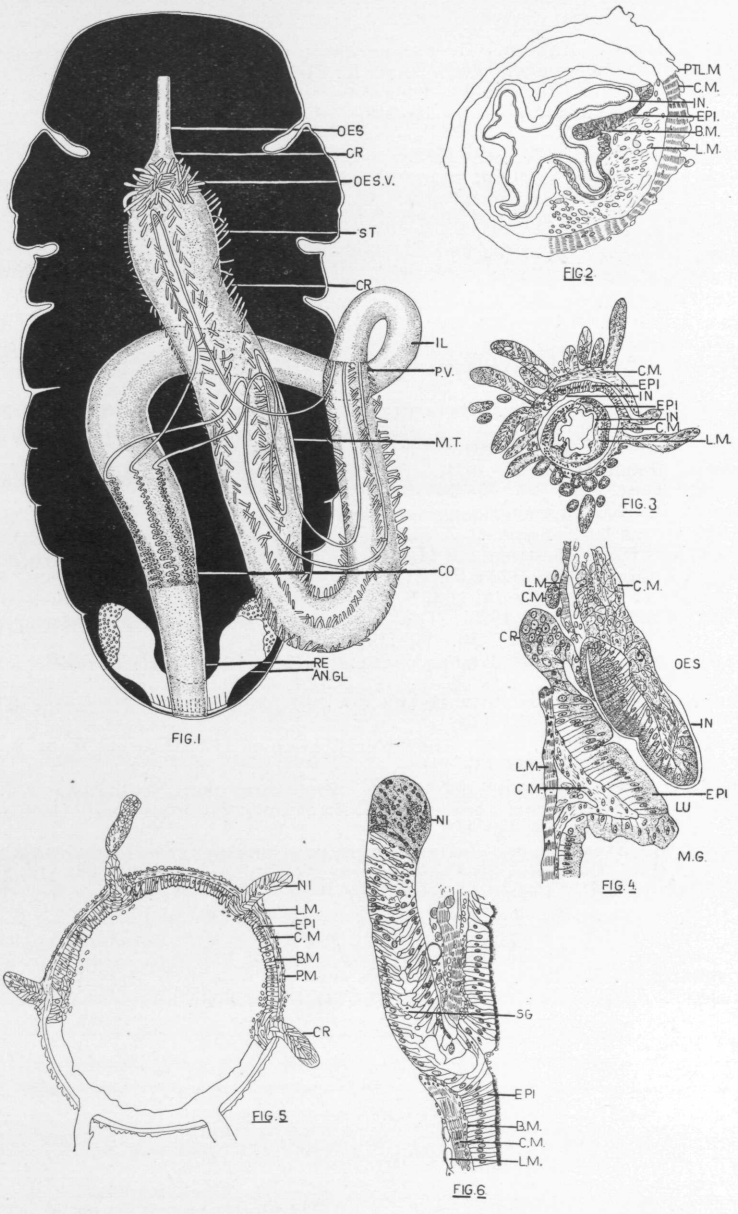


Fig. 1. Dorsal view showing gross dissection of the Alimentary Canal.
Fig. 2. Cross-section through Oesophagus.
Fig. 3. Cross-section through Oesophageal Valve.
Fig. 4. Longitudinal section through Oesophageal Valve.
Fig. 5. Cross-section through anterior mid-gut showing six Crypts.
Fig. 6. Cross-section through anterior mid-gut and a longitudinal section of a crypt (enlarged).

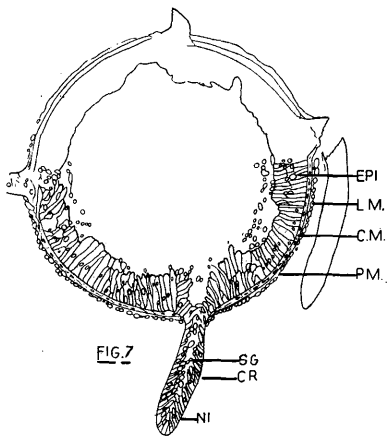


FIG. 7

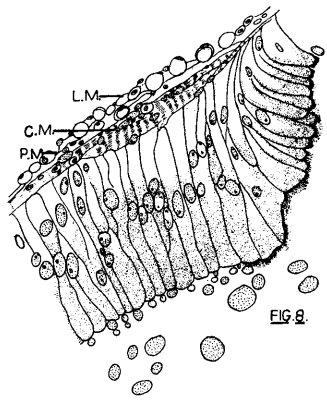


FIG. 8.

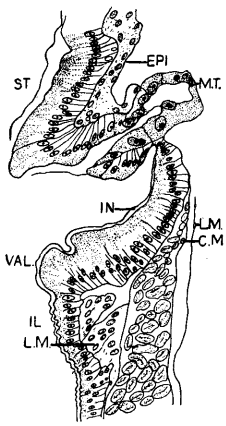
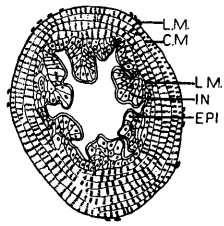


FIG. 9



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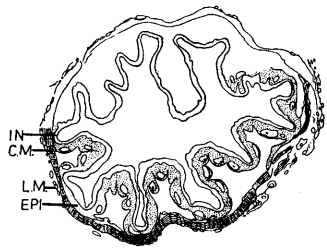

FIG. 11

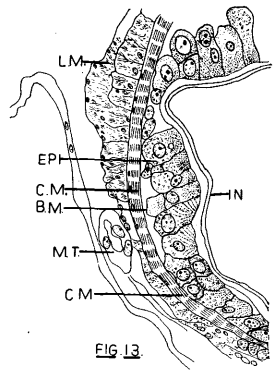

FIG. 13.

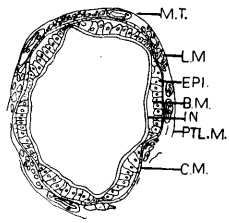

FIG. 12

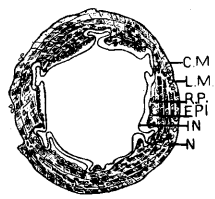

FIG. 14

Fig. 7. Cross-section through posterior mid-gut showing four crypts.
Fig. 8. Enlarged drawing of portion of Fig. 7.
Fig. 9. Longitudinal section showing the pyloric valve.
Fig. 10. Cross-section through the pyloric valve.
Fig. 11. Cross-section through the ileum.
Fig. 12. Cross-section through the colon.
Fig. 13. Detailed drawing of portion of Fig. 12.
Fig. 14. Cross-section through rectum.